Pollen Grain and Microsporogenesis in *Scrophularia Striata* Boiss. (Scrophulariaceae)

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**ABSTRACT**

In this study, pollen morphology and microsporogenesis development of *Scrophularia striata* belong to scrophulariaceae were investigated. The anthers and pollen at developmental different stages were processed for Light and Transmission Electron Microscopy. The results indicated that anthers wall development followed the dicotyledonous type and were tetrascarangeate witch composed of epidermal, endothecium, middle layers and then tapetum layer. In this species, it is shown that the tapetum cells possess in all developmental stages characteristics of the secretory type. Tapetum layer cells have a long stability layer and are uninucleate. Evidence is supplied that the microsporogenesis in *S.striata* can be considered as simultaneous. The microspore tetrads have a tetrahedral arrangement. Mature pollen grains are shed at bicalcular stage. Although microsporogenesis and pollen grains development features plant of the species, *S.striata*, bear many similarities with the Scrophulariaceae, but in view of the type of tapetum layer cells, the pollen grains and tetrad microspore tetrahedral with there are remarkable different other plants in this different.

**INTRODUCTION**

Plant *Scrophularia striata* belong to Scrophulariaceae is the most important medicinal plants. This family has three subfamilies, 222 genera and 4480 species in the world [1]. This genus contains 60 species and subspecies in annual, biennial and perennial of the 28 species that are endemic to Iran [2].

The characteristics of a typical *Scrophularia* include: asymmetrical mostly tubular flowers, ovariety with axile placentation and numerous ovules, capsular fruits, and seeds with endosperm, each are shared with one or several related families [3].

Studies of pollen development and associated changes in the anther yield valuable characters for investigating critical evolutionary changes in the reproductive biology of seed plants, as well as for assessing evolutionary relationships. The development of the male gametophyte involves a series of events culminating in the production and release of mature pollen grains from anthers [4].

Microsporogenesis and pollen grain development have been a focus of interest since generative reproduction in plants depends on pollen structure and function. Proper organization of the microtubular cytoskeleton during meiotic and mitotic divisions is essential for the formation of high quality, viable pollen grains. Disturbances decrease the chance of effective pollination and fertilization of maternal plants [5].

Anther structure in higher plants is very complex and the development is rapid. The four layers of the anther wall display various morphologies and structures after differentiation. Pollen development through the two mitotic cycles is also a rapid process. Recent studies on pollen of seven species belonging to three genera *Kickxia, Scrophularia* and *Veronica* dim light microscope has been done [6].

Present research is the first study on microsporogenesis and pollen morphology of Iranian indigenous species of *Scrophularia*. This paper offers a partial remedy to this situation by providing an account of anther wall, tapetum, microspore, male gametophyte development and pollen morphology in *S.striata*. The main aim of this paper is to present a detailed study on microsporogenesis and pollen morphology of *S.striata*. Because having a few studies on pollen morphology, there is not any information about *S.striata* microsporogenesis. The present paper is the first report and is an attempt to understand the microsporogenesis, anther and pollen grains development in *S.striata* and its taxonomic significance.

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MATERIAL AND METHODS

The flowers and buds in different of developmental stage were collected between 7:00-9:00 AM, during the period 9th March-April in years 2012-2013 from Ilam Salehabad in western of Iran. Flowers were fixed in FAA70 (Formalin, glacial acetic acid and ethanol, 5:5:90v/v). After the fixation process; the voucher specimens were dehydrated during alcohol series, and embedded in paraffin after the process of paraffin saturation in toluene. With rotary microtome, sections of 8-10 μm thickness were taken from the materials embedded in paraffin. Staining was carried out with Eosin according to protocol suggested by Yeung [7] and contrasted Meyer’s Hematoxylin. Several sections for each anther developmental stage were studies under a Zeiss Axiostar plus light microscope. For each stage, at least 20 flowers were studied and photomicrographs were made from the best ones.

For Transmission Electron Microscopy (TEM), anthers at different developmental stages were prefixed overnight in 3% glutaraldehyde in Cacodylate buffer (pH 7.2) at 2 °C for 2 h and then post-fixed in OSO4 at 2°C in the same buffer for 3h. They were dehydrated in an ethanol series (30–100%), and embedded in Epon’s resin. Fine sections were prepared using a Leica Ultracut UCT, and stained with uranyl acetate (1%) for 15 min and followed by lead citrate (6%) for 3 min. The sections were observed and photographed using a JEM-100CXII transmission electron microscope [8].

Results:
Anther and pollen development::

The flowers of *S.striata* are bisexual asymmetrical. The five sepal often persistent are ovate, green, equally sized and coriaceous. The Corolla is generally violet or red, sympetalous, often 2-lipped. The stamens four didynamous and anther locules (personal observation). In sporogenous tissue, the cells are polygonal and tightly appressed, completely filling the locular space. During the early sporogenous stage, the tapetum is undifferentiated. In a cross section of the anther, microspore mother cells (MMC) of *S.striata* are angular in shape and possess a large nucleus with a darkly staining nucleolus (Fig. 1A).

Primary sporogenousa cells were developed directly as microsporocytes. Although the anther ontogeny and microsporogenesis in *S.striata* is a continuous process for descriptive purposes of the structure and ultra-structural details, we distinguished five stages of development: microspore mother cell stage followed by meiosis, tetrads, free young microspores, vacuolated pollen, and mature pollen grains at dehiscence.

Stage 1: Microspore mother cell (MMC):

The young anther wall in *S.striata* consists of four layers from outer to inner, an epidermis, endothecium, one middle layer and tapetum (Fig.1A). Microspores and pollen grains are produced from microspore mother cells (Microsporocytes), MMCs within loculus anthers (pollen sacs) of the flower. These cells are closely arranged in the entire pollen chamber and further develop into microspore mother cell. On anther cross section, microspore mother cells are polygonal, tightly packed, and large in volume, and have large nuclei, prominent nucleoli, but not cytoplasmic vacuoles (Fig. 1B). In this species, the middle layer is a few developed. Epidermal cells show the tangential external wall is thickened. The epidermis in *S.striata* preserves its vitality until the period of anther splitting. One basic type of tapetum has secretory type is visible in this species (Fig. 1A).

![Fig. 1: Scrophularia striata, anther transversal section under a light microscope stained with H&E. (A) PMCs at premeiotic stage and anther wall layers, epiderm (Epi), Endothecium (En) and tapetum (T) Obj 4X. (B) Microspore mother cells (MMC) Obj 40X.](image-url)
Stage 2: Microspore tetrads:

Each microsporocyte undergoes meiosis and resulted in a microspore tetrad (Fig. 2E, 2F). Meiosis staggering including prophase I, metaphase I, anaphase I and then telophase I (Fig. 2B↑) which microspore dyads have resulted. Followed by prophase II, metaphase II, anaphase II (Fig. 2D) and telophase II (Fig. 2B↓) were observed clearly in the specimens. After meiosis II numerous tetrads of microspores appear (Fig. 2E, 2F). Cell wall was not formed between the two newly formed nuclei in telophase I stage (Fig. 2B↑). In the all tetrads, the cytokinesis was done as simultaneous type (Fig. 2E, 2F). The final tetrads are recognized mostly as tetrahedral (Fig. 2E, 2F). Callosic wall is formed around the tetrad and between each monad (Fig. 2E, 2F). In this stage, the tapetal cells are uninucleate and more vacuolated (Fig. 2E). In the two neighboring pollen sacs, microspores development is synchronized (Fig. 2E). During meiosis, the cells of endothecium layer are tangentially elongated and have spiral thickenings which are made of lignin. At the time of dehiscence, the endothecium is still intact the anther connective varies from round to flat (Fig. 2E).

Fig. 2: (A): Section of single loculuse showing early microspore mother cell (MMC) in S. striata. The loculuse is lined by epiderm (E), Endothecium (En), middle layer (ML) and tapetum (T) Obj 4X. (B): Section of single loculuse showing telophase meiosis I↑ (dyad) and anaphase meiosis II↓ (tetrad) Obj 10X. (C): Telophase meiosis II showing early tetrads, special wall (SW) Obj 40X. (D), callosic wall are visible that surrounding early meiosis stages Obj 40X. (E) Section of single loculuse showing anaphase and telophase meiosis II Obj 4X. (F) Tetrahedral tetrads in the anther loculus. Special wall(SW). Callosic wall are visible that surrounding tetrads Obj 40X.

Stage 3: free young microspores:

At this stage, the wall surrounding the four microspore cells dissolve and the microspores in the pollen sac are released. After the dissolution of the callose wall, the sporopollenin wall begins to form. Three different electron- dense layers form the pollen grain wall: ectexine with interrupted tectum, columellae and foot layer; endexine and intine still in formation. The microspores when released from tetrad are non-vacuolated. They
have a dense cytoplasm with irregular shape filled with numerous small vesicles, oil body, abundant amyloplasts and a prominent centrally placed nucleus (Fig. 3A, 3B).

**Stage 4: microspores vacuole with a developed exine wall:**

The microspores have a conspicuous nucleus and their cytoplasm is limited to a parietal position due to the presence of a large vacuole. Its nucleus takes up a peripheral position together the central vacuole develops, i.e., forming a large vacuole squashes the cytoplasm and the nucleus toward the microspore margin. Many mitochondria, proplastid and large lipidic globules are observed in the microspores cytoplasm (Fig. 4). Ubisch bodies and exine have the same high electron-density. Lipidic substances are seen among the exine columellae (Fig. 4). The exine wall of the microspores is formed by a basal layer, numerous columellae close to each other, and a thick tectum (Fig. 4).

**Stage 5: Mature pollen grain:**

Nucleus of microspores then undergoes mitosis and resulted to form two unequal nuclei, a large vegetative and small generative one thus to form binucleate pollens. The generative cell acquires a fusiform aspect with a lobulate outline (Fig. 5). This cell has an electron-translucent wall with membranous inclusions. The generative cell cytoplasm is highly reduced when compared to the large nucleus. The vegetative cell cytoplasm is very dense, filled with numerous small vesicles, mitochondria, lipidic globules, amyloplasts and ERr with extended cisternae. The vegetative cell nucleus is lobed, for this reason, small portions of it are seen in the fine sections. Pollen grains have a considerable thick exine. Pollen grains are two-celled when shed (Fig. 5).
The exine is more compacted. It has collumella on a thick basal layer and conspicuous supractectum spines. The exine has a very thin endexine layer, and a fibrilar intine with numerous invaginations coated by the plasmalemma can be observed. Some tapetal derives are left between the collumella (Fig. 5).

Inside the vegetative cell cytoplasm there can be found extensive cisterns of the ERr, numerous mitochondria and abundant amyloplasts. The former possesses a conspicuous nucleus and a reduced cytoplasm with scarce mitochondria and bodies with concentric intravacular membranes. It presents a thin, irregular wall that is transparent to electrons. The generative cell is surrounded by numerous vesicles with a fibrilar content of low electron density in contact with more and smaller vesicles (Fig. 5).

The anther of *S. striata* is tetrasporangiate (Fig. 6A). In this species the mature pollens are bicellular and ellipse shape. The endothecial cells are generally uninucleate (Fig. 6B).

**Discussion:**

This study is the first comprehensively report about pollen grains ontogeny in relation with pollen development in the genus *Scrophularia* in Iran. The pollen grain development followed the normal pattern of the development described for most of the Angiosperms [9]. The anther wall development in scrophulariaceae was reported as dicotyledonous type (10), including *Verbascum* [11]. The presented results confirmed that anther walls of *S. striata* also belonged to Dicotyledonous type.

A few studies performed about anther and pollen morphology of other genus Scrophulariaceae in the world but pollen morphology such as *S. striata* has not been examined. The consequences of our study are consistent with the results of anther and pollen morphological investigation of other Scrophulariaceae genus.

The undifferentiated anther of *S. striata* was ovoid-shaped and tetrasporangiated. Furthermore periclinal divisions of sporogenous cells resulted in the formation of pollen mother cells in *S. striata*. The researchers stated that at early developmental stages the epidermal, endothecial and middle layer cells had prominent nuclei, large vacuoles, numerous rough endoplasmic reticula and mitochondria. These characters are consistent with [12].
Analysis of *S. striata* has shown that the endothelial thickenings started to accumulate in inner tangential and radial wall at vacuolated pollen stage. The middle layer degenerated at young microspore stage. The tapetum has been considered to be the nutritive tissue for the developing pollen. The major function of the tapetum is to provide essential metabolites to the development of the microspores [13]. In the secretory tapetum metabolites in the form of soluble carbohydrates, amino acids and peptides are released into the loculus from which they are taken up by the developing pollen [14]. The uninucleate tapetal cells were commonly observed. The cells of secretory tapetum maintain their position. In *S. striata*, the uninucleated tapetal cells developed at young microspore stage. The results indicated that microsporogenesis in the *S. striata* is simultaneous. Microsporogenesis can be described as simultaneous if cytokinesis and callose deposition both occur after meiotic divisions are complete. Simultaneous microsporogenesis results in tetrads that are held in a tetrahedral configuration [15].

In the *S. striata*, proteins are located in exine cavities (tectate grain) as pollenkitt or trypine. It has been reported that [11] orbicules originate in the cytoplasm of the tapetal cells as lipoidal pro-orbicular bodies that accumulate below the membrane and eventually extrude to the cell surface (facing the locule) where they provide sporopollenin precursors for exine formation.

The endexine is the inner part of the exine, is located above the foot layer and has a different electron density than the foot layer. These data are consistent with Punt et al., [15] and Saeidi & Zarrei [16] for *Veronica*.

At the microspore mother cell (MMC) stage in the *S. striata* numerous cytoplasmic connections are observed while the callose wall starts to form and the primary wall is still present. The existence of such connections has been reported for many angiosperms [17]. Liu and Huang [18] showed that the foot layer and endexine began formation simultaneously in *Caesalpinia* and *Uraria*. In the present study, the foot layer and endexine are also formed simultaneously and concomitant with the callose wall dissolution.

In the *S. striata*, mitochondria and ribosome found on the cytoplasm of the microspores suggest great metabolic activity. Due to the activity of the numerous vesicles in the cytoplasmic periphery are present. This indicates the possible role of vesicles in the production of callose. These properties accordance with[19].

In *S. striata*, Microspore mitosis is an asymmetric division. This division can also be viewed as a determinative division in that the resulting generative and vegetative cells have very different cell fates. The generative cell of the mature pollen grain is enclosed by the vegetative one. These data are reported by [20].

At the free microspore stage, a material with a similar electron density to sporopollenin fills the whole locule and deposits over the pollen grain wall. Similar observations have been recorded in *Passiflora ssp*. [12]. Such material might be considered to be one of the sporopollenin precursors originated by the tapetum [20].

In this species the pollen grains are two-celled when shed. Because of a lake of sufficient data about microsporogenesis from *Scrophularia*, we have not compared the microsporogenesis characters of *S. striata* with those of many other species.

Although microsporogenesis and pollen grains development features plant of the species, *S. striata*, bear many similarities with the Scrophulariaceae, but in view of the type of tapetum layer cells (Secretory), the pollen grains (oval shape) and tetrad microspore tetrahedral with there are remarkable different other plants in this different.

We hope the present study provides some new insights for interpreting the evolution of anther and pollen development as well as the compound pollen in the *S. striata*. More ontogenetical studies are needed to fully understand the great diversity of anther and pollen characters in the Scrophulariaceae.

**REFERENCE**


